Phospholipid-Dependence of Oestrone UDP-Glucuronyltransferase and *p*-Nitrophenol UDP-Glucuronyltransferase

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Hepatic UDP-glucuronyltransferase activity was resolved into two fractions, one exhibiting oestrone glucuronyltransferase activity and the other exhibiting *p*-nitrophenol glucuronyltransferase activity. Hydroxyapatite-column chromatography removed greater than 95% of the phospholipids from both preparations. The partially purified delipidated enzymes were essentially devoid of catalytic activity, but activities were restored by the addition of phospholipids or phosphatidylcholine mixtures containing various saturated and unsaturated fatty acids. Both oestrone and *p*-nitrophenol glucuronyltransferase activities were reconstituted to similar degrees with the phosphatidylcholine mixtures. When purified phospholipids were tested, phosphatidylcholine and lysophosphatidylcholine were most effective in restoring activity, whereas phosphatidylethanolamine was the least effective. These results further suggest that oestrone and *p*-nitrophenol UDP-glucuronyltransferases are dependent on phospholipids for their activity.

The catalytic activity of many membrane-bound enzymes is dependent upon the presence of phospholipids (Martonosi *et al.*, 1968; Strobel *et al.*, 1970; Cater *et al.*, 1975; Gazzotti *et al.*, 1975). Similarly, a phospholipid requirement has been suggested for UDP-glucuronyltransferase (EC 2.4.1.17) activity. Treatment with phospholipases of microsomal fractions expressing fully active UDP-glucuronyltransferase activity results in a decreased rate of glucuronidation of *p*-nitrophenol (Graham & Wood, 1969; Atwood *et al.*, 1971; Berry *et al.*, 1976, 1978; Graham *et al.*, 1977). Phospholipids added to these preparations restored the *p*-nitrophenol glucuronyltransferase activity. Jansen & Arias (1975) reported that gel filtration of solubilized microsomal preparations chromatographed in the presence of detergents leads to delipidation and consequently inactivation of bilirubin and *p*-nitrophenol glucuronyltransferase activities. Addition of phospholipids to fractions eluted from the columns resulted in the restoration of transferase activity of the bilirubin enzyme but not of the *p*-nitrophenol enzyme. Subsequently, Graham *et al.* (1977), by using a similar gel-filtration method to remove phospholipids, was able to restore *p*-nitrophenol glucuronyltransferase activity if the detergent in the preparation was removed by Bio-Beads in the presence of phospholipids. Rao *et al.* (1972) have shown that phospholipids are capable of restoring

oestriol glucuronyltransferase activity to a partially purified microsomal preparation that had been treated with phospholipase C. These results suggest that the catalytic activity of glucuronyltransferase is phospholipid-dependent. However, more conclusive studies need to be performed with preparations that are solubilized, purified and free of lipid.

Recently, our laboratory has reported the separation and purification of morphine and *p*-nitrophenol glucuronyltransferase activities (del Villar *et al.*, 1975, 1977) and the separation and purification of oestrone and *p*-nitrophenol glucuronyltransferase activities (Tukey *et al.*, 1978). Bock *et al.* (1977) have demonstrated the separation and purification of 1-naphthol and morphine glucuronyltransferase activities. Gorski & Kasper (1977) and Burchell (1977) achieved considerable purification of a UDP-glucuronyltransferase activity, but confined their studies to *p*-nitrophenol glucuronyltransferase activity. Gorski & Kasper (1977) reported a phospholipid content of 25.2 μg of P_i /mg of protein in their purified preparation, but Burchell & Hallinan (1978) observed a content of about 0.3 μg of P_i /mg of protein in a highly purified preparation. The former group did not study phospholipid requirements of that preparation and the latter group used a preparation that contained Lubrol 12A9, a detergent that itself may support activity in a manner similar to phospholipids. The present studies were designed to solubilize, further purify and delipidate UDP-glucuronyltransferase activity to-

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ward oestrone and *p*-nitrophenol and to investigate the possible requirements for phospholipids in the catalytic activity of these resolved enzymes.

Experimental

Materials

[4-¹⁴C]Oestrone, and Aquasol 2 were purchased from New England Nuclear, Boston, MA, U.S.A. *p*-Nitro[U-¹⁴C]phenol was purchased from California Bionuclear Corp., Sun Valley, CA, U.S.A. Synthetic didodecanoyl phosphatidylcholine (C_{12:0}), ditetradecanoyl phosphatidylcholine (C_{14:0}), dihexadecanoyl phosphatidylcholine (C_{16:0}), dioctadecanoyl phosphatidylcholine (C_{18:0}), dilinoleoyl phosphatidylcholine (C_{18:2}), diarachidonyl phosphatidylcholine (C_{20:4}), phosphatidylcholine (bovine), lyso-phosphatidylcholine (egg) and phosphatidylethanolamine (bovine) were purchased from Supelco Inc., Bellefonte, PA, U.S.A. UDP-glucuronic acid, *p*-nitrophenol and oestrone were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. DE-52 DEAE-cellulose was obtained from Whatman Biochemicals, Clinton, NJ, U.S.A., and Sephadex G-25 was obtained from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. Emulgen 911 was purchased from Kao-Atlas, Tokyo, Japan. Silicic acid (Unicil) was obtained from Clarkson Chemical Co., Williamsport, PA, U.S.A. T.l.c. plates (silica-gel GF, 250 μm) were purchased from Analtech Inc., Newark, DE, U.S.A. Hydroxyapatite was purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. All other chemicals were analytical-grade reagents.

Methods

Oestrone and *p*-nitrophenol glucuronyltransferase assays. Both oestrone and *p*-nitrophenol glucuronyltransferase activities were assayed as previously described (Tukey *et al.*, 1978). Oestrone glucuronyltransferase activity was determined by incubating the enzyme in 0.05 M-Tris/HCl buffer, pH 8.0, with 0.01 M-MgCl₂ and 10 μM-[4-¹⁴C]oestrone (sp. radioactivity 9000 d.p.m./nmol) in a total volume of 1 ml. The *p*-nitrophenol glucuronyltransferase activity was assayed by incubating enzyme in 0.1 M-Tris/HCl buffer, pH 8.0, with 0.01 M-MgCl₂ and 1 mM-*p*-nitro-[U-¹⁴C]phenol (sp. radioactivity 105 d.p.m./nmol) in a total volume of 2 ml. Reactions were initiated by adding the enzyme solution, except when conducting the reconstitution experiments, in which case the reactions were started by adding UDP-glucuronic acid (see below). Control incubations were carried out under the same conditions by using all components, except that UDP-glucuronic acid was omitted.

Separation of oestrone and *p*-nitrophenol glucuronyltransferase activities. A detailed description of this procedure has been published (Tukey *et al.*, 1978), but for clarity a brief resumé is included.

Rabbit liver microsomal fraction was solubilized with sodium cholate, centrifuged at 100 000g for 2 h, and the supernatant fraction treated with solid (NH₄)₂SO₄. The precipitate that results from 35–70% (NH₄)₂SO₄ saturation was collected and dissolved in 25 mM-Tris/HCl buffer, pH 8.0, containing 20% (v/v) glycerol, 0.1 mM-dithiothreitol and 1 mM-EDTA (buffer A). It was immediately chromatographed on a Sephadex G-25 column. The eluate was dialysed and it was diluted to a concentration of 10 mg of protein/ml, essentially removing all cholate. Emulgen 911 was added (0.1 mg/mg of protein) to the solution, which was then applied to a DE-52 DEAE-cellulose column (2.5 cm × 100 cm) previously equilibrated in the same buffer (buffer B). This procedure resolved the transferase activities into two fractions. The void volume contained greater than 90% of the oestrone glucuronyltransferase activity. By using a linear 0–0.5 M-KCl gradient, the remaining oestrone glucuronyltransferase activity and the majority of the *p*-nitrophenol glucuronyltransferase activity were eluted at approx. 0.2 M-KCl. Complete separation of the two activities was accomplished by chromatographing each fraction separately on another DE-52 DEAE-cellulose column (1.5 cm × 30 cm) previously equilibrated in 15 mM-Tris/HCl buffer, pH 8.0, containing 20% (v/v) glycerol, 0.1 mM-dithiothreitol, 1 mM-EDTA and 0.1% (v/v) Emulgen 911 (buffer C). The oestrone glucuronyltransferase activity was eluted in the void volume, whereas the *p*-nitrophenol glucuronyltransferase activity bound to the column and was eluted with a linear 0–0.2 M-KCl gradient.

Hydroxyapatite chromatography of oestrone and *p*-nitrophenol glucuronyltransferase activities. The resolved fractions after rechromatography on DE-52 DEAE-cellulose column were concentrated by Amicon ultrafiltration (Amicon PM10 membrane), dialysed for 24 h with three changes of 5 mM-Tris/HCl buffer, pH 8.0, containing 20% (v/v) glycerol, 0.1 mM-dithiothreitol, 1 mM-EDTA and 0.1% Emulgen 911 (buffer D). Each fraction was applied separately to hydroxyapatite columns (1.4 cm × 4 cm) and washed with approx. 100 ml of the equilibration buffer. The transferases were eluted from the column with a linear 0–0.75 M-KCl gradient. Oestrone and *p*-nitrophenol glucuronyltransferase activities were localized by assaying each fraction in the presence of 50 μg of rabbit liver microsomal phospholipids.

However, in certain experiments the protein retained on the column was washed with 300 ml of a solution containing 10 mM-Tris/HCl, pH 8.0, containing 0.1 mM-dithiothreitol, 1 mM-EDTA and 20% (v/v) glycerol, to decrease the Emulgen 911 concen-

tration. Then 0.02% Emulgen 911 and 0.75 M-KCl were added to this solution and it was used to elute the proteins from the hydroxyapatite columns. This procedure allowed us to test the effects of increasing Emulgen 911 concentration on the oestrone and *p*-nitrophenol glucuronyltransferase activities in the presence of various concentrations of phospholipids. Attempts to elute the protein with KCl in the absence of Emulgen were unsuccessful.

Extraction of lipids. Lipids were extracted by the method of Folch *et al.* (1957). Chloroform/methanol (2:1, v/v) was added to each fraction (40:1, v/v) and stirred overnight at 4°C. This mixture was filtered through Whatman no. 1 paper into a separating funnel, 0.1 M-KCl (15%, v/v) was added, and the solutions were allowed to separate at 4°C overnight. The lower phase was evaporated to dryness, resuspended in 4 ml of chloroform and applied to a silicic acid column (1.0 cm × 30 cm). Neutral lipids, glycosphingolipids and phospholipids were separated by passing through the column, in sequence, 100 ml of chloroform, 100 ml of acetone/methanol (9:1, v/v) and 200 ml of methanol. The individual phospholipids were separated as described by Ames (1968). Each fraction was evaporated to dryness and stored at -40°C in a small volume of chloroform/methanol (2:1, v/v).

Phospholipid fatty acid composition. Methyl esters of the fatty acids were prepared from the isolated phospholipids by transesterification (Morrison & Smith, 1964). The methyl esters were analysed with a computerized Hewlett-Packard A gas chromatograph equipped with dual-flame ionization detectors. The analysis was conducted with a 6 ft (1.83 m) glass column packed with 10% SP-2340 on Chromosorb AW (100-120; Supelco) as described by Kehrer & Autor (1977). Methyl linolenate was used as the internal standard.

Dispersion of phospholipids. Phospholipids were microdispersed by ultrasonication in 1-2 ml of buffer D. Each sample was maintained at 4°C and ultrasonicated at intervals of 30 s for 5 min. However, in experiments in which the effects of Emulgen 911 on transferase activities were examined, phospholipids were dispersed in water. In a standard assay for oestrone and *p*-nitrophenol glucuronyltransferase activities, all assay components except UDP-glucuronic acid were combined with various amounts of lipid and enzyme and incubated at room temperature for 15 min. Each reaction was then initiated by adding UDP-glucuronic acid.

Phosphorus determination. Lipid phosphorus was assayed by the method of Bartlett (1959). When column eluates were assayed for phosphorus content, 1 ml of each sample was extracted with 30 ml of chloroform/methanol (2:1, v/v) and filtered through Whatman no. 1 paper. To remove buffer components

such as glycerol and detergent, 3 ml of 0.1 M-KCl was added and each tube was shaken gently. The samples were centrifuged at 1500 rev./min for 15 min, the aqueous phase was decanted and the organic phase washed twice with 5 ml of the upper phase. The organic phase was evaporated to dryness and assayed for lipid phosphorus.

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* (1951), and a modification of this assay was used when buffer components interfered with the determination (Tukey *et al.*, 1978).

Results

Separation of UDP-glucuronyltransferase activities and phospholipids by DEAE-cellulose chromatography

Rabbit liver UDP-glucuronyltransferase activities, one exhibiting activity towards oestrone and the other exhibiting activity towards *p*-nitrophenol, were resolved into two separate fractions by column chromatography on DE-52 DEAE-cellulose (Tukey *et al.*, 1978). The void volume contained the majority of the phospholipids, oestrone glucuronyltransferase activity and a small amount of the *p*-nitrophenol glucuronyltransferase activity. The majority of the *p*-nitrophenol glucuronyltransferase activity, the remaining oestrone glucuronyltransferase activity and residual phospholipids were eluted later with a 0-0.5 M-KCl gradient. When these fractions were rechromatographed on a second DE-52 DEAE-cellulose column, each activity was eluted as a single peak with the same elution profile as the first column (Tukey *et al.*, 1978). After rechromatography, the oestrone glucuronyltransferase fraction contained more phospholipid (Table 1) (expressed as μg of phosphorus/mg of protein) than after elution from the first column, indicating that the phospholipids were being concentrated in the void volume. The fractions from the second DE-52 DEAE-cellulose column that contained *p*-nitrophenol glucuronyltransferase activity had less than 7% of the initial amount of the lipid phosphorus (Table 1). Addition of microsomal phospholipids to either the *p*-nitrophenol or oestrone enzyme fractions did not result in increased activity.

Removal of phospholipids by hydroxyapatite-column chromatography

After elution of the oestrone and *p*-nitrophenol glucuronyltransferase activities from the DE-52 DEAE-cellulose columns, both fractions were concentrated, dialysed and applied separately to hydroxyapatite columns. The phospholipids from either enzyme preparation were eluted from the column with the equilibration buffer, but the protein remained firmly bound to the hydroxyapatite. The protein

Table 1. *Phospholipid fatty acid and phosphorus analysis*

After isolation of phospholipids at the various steps during the purification and separation of oestrone and *p*-nitrophenol UDP-glucuronyltransferase activities, phosphorus and fatty acids were determined. (A) represents μg of fatty acids or phosphorus/mg of protein associated with the oestrone UDP-glucuronyltransferase activity, and (B) represents μg of fatty acids or phosphorus/mg of protein associated with the *p*-nitrophenol UDP-glucuronyltransferase activity.

Purification step	Fatty acid ($\mu\text{g}/\text{mg}$ of protein)										Phosphorus ($\mu\text{g}/\text{mg}$ of protein)	
	$\text{C}_{16:0}$		$\text{C}_{18:0}$		$\text{C}_{18:1}$		$\text{C}_{18:2}$		$\text{C}_{20:4}$			
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Solubilized microsomal preparation	17.64		29.69		8.29		31.24		9.45		18.1	
35–70% satd.– (NH_4) ₂ SO ₄ precipitate	7.29		10.43		3.37		10.59		2.12		6.0	
First DEAE-cellulose chromatography	18.74	1.43	25.32	4.81	8.34	1.04	38.22	3.28	16.75	1.03	8.3	1.24
Second DEAE-cellulose chromatography	25.98	0.49	33.28	1.17	11.17	0.27	51.87	0.87	21.59	0.52	15.6	0.44
Hydroxyapatite chromatography	0.66	0.14	0.86	0.29	0.29	0.06	0.26	0.39	0.09	0.16	0.62	—*

* No detectable amount.

Fig. 1. *Re-activation of oestrone UDP-glucuronyltransferase activity with different phospholipids*

After hydroxyapatite chromatography, the delipidated fractions containing oestrone UDP-glucuronyltransferase activity were assayed in the presence of variable amounts of phospholipids. (a) Phosphatidylcholines with different fatty acid substituents were present as follows: $\square$, C_{12:0}; $\blacktriangle$, C_{14:0}; $\triangle$, C_{18:2};

associated with either oestrone or *p*-nitrophenol glucuronyltransferase activities was eluted with a linear 0–0.75 M-KCl gradient in buffer D at a concentration of about 0.3 M-KCl. The phospholipid phosphorus or fatty acids associated with each enzymic activity are reported in Table 1. About 95% of the phospholipid phosphorus and fatty acids associated with the oestrone glucuronyltransferase that is applied to the column is removed from the protein. Although phosphorus was undetectable in the fraction containing *p*-nitrophenol glucuronyltransferase activity after hydroxyapatite chromatography, analysis of phospholipid fatty acid content revealed that chromatography on hydroxyapatite efficiently removed phospholipids from this preparation also.

Before hydroxyapatite chromatography the specific activity of oestrone UDP-glucuronyltransferase activity was 15 nmol conjugated/min per mg of protein and the specific activity of the *p*-nitrophenol glucuronyltransferase activity was 358 nmol conjugated/min per mg of protein. After elution of these proteins with KCl, the activities were nearly undetectable, but were restored when each enzyme was assayed in the presence of microsomal phospholipid. The recovery of oestrone and *p*-nitrophenol glucur-

$\circ$, C_{16:0}; $\bullet$, C_{20:4}; $\blacksquare$, C_{18:0} (see under 'Materials' for full names). Other phospholipids were tested in (b) as follows: $\triangle$, microsomal phospholipids; $\square$, lysophosphatidylcholine; $\blacksquare$, phosphatidylcholine; $\bullet$, phosphatidylethanolamine. All assays were run in the presence of 30 μg of protein in a total volume of 1 ml (see under 'Methods' for complete details).

onyltransferase activities and protein was greater than 70% of the total activity or protein that was applied to the hydroxyapatite columns.

Restoration of enzyme activity

The abilities of various phospholipids to restore oestrone and *p*-nitrophenol glucuronyltransferase activities resolved by hydroxyapatite chromatography are presented in Figs. 1 and 2. All of the phospholipids tested reactivated both oestrone and *p*-nitrophenol glucuronyltransferase activities, albeit each to different degrees. Microsomal phospholipids, phosphatidylcholine (bovine) and lysophosphatidyl-

choline (egg) were able to re-activate the oestrone glucuronyltransferase activity to the value of the specific activity of the preparation before application to the hydroxyapatite column (Fig. 1b). Phosphatidylethanolamine was the least effective in this regard. The re-activation curves of oestrone glucuronyltransferase activity with different phosphatidylcholines are plotted in Fig. 1(a). The unsaturated phosphatidylcholine, dilinoleoyl phosphatidylcholine ($C_{18:2}$), was the most effective, followed by diarachidonoyl phosphatidylcholine ($C_{20:4}$). Of the saturated phosphatidylcholines, didodecanoyl phosphatidylcholine ($C_{12:0}$) was more effective than ditetradecanoyl phosphatidylcholine ($C_{14:0}$) and dihexadecanoyl phosphatidylcholine ($C_{16:0}$) and dioctadecanoyl phosphatidylcholine ($C_{18:0}$) was relatively ineffective. Unlike oestrone UDP-glucuronyltransferase activity, activity towards *p*-nitrophenol was activated by lysophosphatidylcholine and phosphatidylcholine to a much greater extent than by microsomal phospholipids (Fig. 2b). Phosphatidylethanolamine again was the least effective.

p-Nitrophenol glucuronyltransferase activity was restored in a similar fashion by both saturated and unsaturated phosphatidylcholines (Fig. 2a). With the unsaturated phosphatidylcholines, dilinoleoyl phosphatidylcholine ($C_{18:2}$) was the most effective, followed by a small decrease in maximal enzymic activity as the number of double bonds is increased [dilinoleoyl phosphatidylcholine ($C_{18:2}$) and diarachidonoyl phosphatidylcholine ($C_{20:4}$)]. Similarly, with the saturated phosphatidylcholines, the greatest re-activation was achieved with didodecanoyl phosphatidylcholine ($C_{12:0}$), followed by ditetradecanoyl phosphatidylcholine ($C_{14:0}$) and dihexadecanoyl phosphatidylcholine ($C_{16:0}$), and the least effective was dioctadecanoyl phosphatidylcholine ($C_{18:0}$).

When phosphatidylcholine and phosphatidylethanolamine were isolated from rabbit liver microsomal fractions and incubated with the delipidated transferases, the restoration of enzyme activity paralleled the increase that was observed when the transferases were treated with bovine phosphatidylcholine and egg-white phosphatidylethanolamine (results not shown). Other lipid fractions that were isolated from microsomal fraction by silicic acid chromatography, such as glycosphingolipids and neutral lipids, were unable to reconstitute either the delipidated oestrone or *p*-nitrophenol glucuronyltransferase activities.

Effect of Emulgen 911 on phospholipid restoration of transferase activities

To determine whether phospholipids were merely reversing an inhibition of transferase activities produced by Emulgen 911, experiments were designed to test the effect of phospholipids in the presence of

Fig. 2. Reactivation of *p*-nitrophenol UDP-glucuronyltransferase activity with different phospholipids

After hydroxyapatite chromatography, the delipidated fractions containing *p*-nitrophenol UDP-glucuronyltransferase activity were assayed in the presence of various amounts of phospholipids. The phosphatidylcholines (a) and other phospholipids (b) were assayed in the presence of 40 μ g of protein in a total volume of 2 ml (see under 'Methods' for complete details). The symbols in (a) and (b) are defined in the legend to Fig. 1.

various concentrations of Emulgen 911. Transferase preparations were chromatographed on hydroxyapatite and the protein that was bound to the hydroxyapatite was washed extensively with detergent-free buffer as described under 'Methods'. Then the protein was eluted from the column with 0.02% Emulgen 911 in the presence of 0.75M-KCl. In the presence of 0.02, 0.05 and 0.1% Emulgen 911 the curves obtained for phospholipid restoration of either oestrone or *p*-nitrophenol glucuronyltransferase activity were essentially the same as shown in Figs. 1(a) and 2(a). When Emulgen 911 concentration was increased to 0.5%, a 70% inhibition of activity occurred that was not reversed by phospholipids. Therefore there is no concentration-dependent inhibition of transferase activity by Emulgen 911 or an apparent relationship between phospholipid and Emulgen 911 under the conditions implemented for these experiments. Furthermore, when inhibition of transferase activity by Emulgen 911 does occur (at 0.5%), phospholipids did not reverse the inhibition.

Discussion

The present work further strengthens the concept that oestrone and *p*-nitrophenol glucuronyltransferase activities are dependent on phospholipids for their activity. Results were obtained with preparations of solubilized microsomal fractions that were resolved into separate and distinct transferase activities as described previously (Tukey *et al.*, 1978). These preparations were chromatographed on hydroxyapatite columns and phospholipids were removed by low concentrations of Emulgen 911. The elution of proteins was carried out by appropriate concentrations of Emulgen 911 and KCl. In the absence of added phospholipids, glucuronyltransferase activities were very low, but activities could be restored by the addition of various phospholipids. The ratio of phospholipid to protein required for maximal restoration of activity (μg of phospholipid/ μg of enzyme protein) was about 4, a value that remained constant until Emulgen 911 concentrations over 0.1% were used. The restoration of activity was dependent on the phospholipid concentration and the concentration, dependency was not altered by increasing concentrations of the detergent Emulgen 911. These results differ significantly from those of other laboratories in that Emulgen 911 appears not to support glucuronyltransferase activity as do other detergents, such as Lubrol 12A9 (Burchell & Hallinan, 1978).

Lysophosphatidylcholine and phosphatidylcholine were more effective than the phospholipids extracted from the microsomal preparation when *p*-nitrophenol glucuronyltransferase activity was studied, whereas the microsomal phospholipids were somewhat more

effective in restoring the activity of oestrone glucuronyltransferase. Phosphatidylethanolamine was the least potent of all the phospholipids tested, in agreement with previous work by Atwood *et al.* (1971) and Jansen & Arias (1975). For both oestrone and *p*-nitrophenol glucuronyltransferase activities, the restoration curves showed that each synthetic phosphatidylcholine that was used re-activated both enzymes in a similar manner. For saturated phosphatidylcholines, activities increased when the chain length of the fatty acids decreased ($C_{12:0} > C_{14:0} > C_{16:0} > C_{18:0}$). With unsaturated phosphatidylcholines, $C_{18:2}$ was more effective than $C_{20:4}$.

The role of phospholipids in the restoration of glucuronyltransferase activity toward oestrone or *p*-nitrophenol is not known at this time. Billings *et al.* (1978) have shown by isoelectric focusing that the oestrone and *p*-nitrophenol glucuronyltransferase activities exhibit different pI values. These pI values did not change when preparations were studied after hydroxyapatite chromatography to remove phospholipids. Therefore, pI values for oestrone and *p*-nitrophenol glucuronyltransferase activities are not altered by removal of phospholipids. Further studies are required to determine the role of phospholipids in the reaction between the transferase and its substrates.

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